

Atty. Ref.: VTI-114.8B(CIP)

-12-of-27-

#### IV. AMENDMENTS TO THE DRAWINGS

--- Replacement and annotated mark-up drawing sheets for amended figures showing the amended figures, if any, are attached at the Appendix hereto. Each figure is in compliance with 37 C.F.R. § 1.84. An explanation of the changes, if any, is set forth below in this "Amendments to the Drawings" section. Replacement drawing sheets are identified in the top margin as "Replacement Sheet." Any replacement drawing sheet including amended figures includes all of the figures appearing on the immediate prior version of the sheet. Any annotated drawing sheets, if the same are required by the Examiner, are identified in the top margin as "Annotated Marked-Up Drawings." Any deleted figure is noted by an instruction to delete the figure. Any corresponding amendment to the specification necessary to be made because of an amendment to the drawings in this section is made in the corresponding "Amendments to Specification" section.

- THE DRAWINGS OF THE PATENT IS HEREBY AMENDED AS SET FORTH BELOW:
  - *No Amendment Made to the Drawings*
  - *Attachments: None*

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-13-of-27-

## V. REMARKS/ARGUMENTS

- STATUS OF THE CLAIMS

Claims 1 and 4–24 remain pending in this application. Claim 22 is currently amended to correct a minor spelling error and to provide additional limitation. Claims 2 and 3 were previously cancelled. Claims 5, 7 – 13, 15, 17 – 21 and 24 are as in the original application.

- OBJECTIONS

- OBJECTIONS TO CLAIMS

- Examiner's Position

The Examiner has raised no objections.

- Applicants' Response

N/A

- REJECTIONS

- REJECTION UNDER 35 U.S.C. §102(b)

- REJECTION UNDER 35 U.S.C. §102(b)

- Examiner's Position

The Examiner has rejected claims 22-24 under 35 U.S.C. §102(b) based on the disclosure of Karasawa *et al.*, JP 55-079441 reference (as cited paragraph 4, page 2 of the Office Action). Examiner has held that the blue form of methylene blue is known to inherently absorb strongly at 650 nm, and that even similar dye types were shifted a few nm due to the chemical environment, their broad absorption would still include wavelengths within the recited range of the invention. Examiner has indicated the importance of supplementary evidence to substantiate Applicants' arguments that methylene blue does not

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-14-of-27-

meet the requirements of operability, but that propylene blue does meet the requirements of operability of the invention.

- Applicants' Response

Applicants herein address the Examiner's 102(b) rejections of claims 22-24 as set forth in the Examiner's Office Action. Applicants respectfully traverse the rejections of claims 22 – 24 based in part on the argument that each element of the claim embodiments is not found in the prior art of reference, and also that the invention as taught by Karasawa *et al.* is demonstrably inoperative under the conditions required for the instant invention.

Furthermore, Applicants respond authoritatively to Examiner's request for independent evidence relating to the absorption spectrum of methylene blue in a dried film such as that described by Karasawa *et al.*, that was deposited from an aqueous solution. Specifically, Applicants independently demonstrate that under the conditions described by Karasawa *et al.*, methylene blue aggregates, or dimerizes predominantly, and that the resultant dimeric form absorbs light very poorly at the operative wavelength of 650 nm, the wavelength of inquiry cited by the Examiner.

In contrast to Examiner's concern that the dyes of Karasawa *et al.* might be shifted only "...a few nm...", Applicants support their previous observations that under the conditions of the invention, such dyes are blue-shifted roughly 60 nanometers and further that the absorption bands fundamentally changes shape.

Applicants have previously pointed out, at paragraph [0017] of prepublication U.S. 20040152017 that "...the present inventors have also discovered that the dye composition can effectuate a lambda shift, in particular a red shift, if the dye molecules aggregate in the coating. For example, methylene blue which has an absorption at 650 in aqueous medium, is found to absorb at 600 nm in a typical DVD coating. Aggregation may be caused by the stereochemical structure of the dye substance utilized. The effect of the dye system on the overall coating thickness has also been unexpectedly found to affect jitter, wobble and playback fidelity of an optical disc." Applicants have also specifically noted at paragraph [0060] of the prepublication that methylene blue does not provide the absorption characteristics required for an operative invention. In particular, Applicants noted that "A DVD read laser has a spectra centered about 650 nm wavelength. The absorption spectra for

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-15-of-27-

methylene blue in solution shows an absorption maxima at 655 nm. While such dye might appear to be useful in itself as a transient optical state change material, when applied to optical disc it was observed that the absorption underwent a bathochromic shift with the spectrum having an absorption maxima at about 590 nm due to aggregation. The absorption spectrum of the compound was found to be modifiable by altering the steric bulk on the nitrogen...".

In reference to the phenomenon of aggregation of methylene blue, Applicants now draw the Examiner's attention to the newly discovered reference (see Appendix A) "Self-aggregation of Methylene Blue in aqueous medium and aqueous solutions of Bu<sub>4</sub>NBr and Urea" by Patil *et al.*, that provides at Fig. 3 a resolved spectrum of the methylene blue dimer (lower curve), as well as that of methylene blue in the monomer state. Additionally, the authors were able to measure a dissociation constant, K, for the methylene blue dimer state. The value of the dissociation constant determined therein was  $2.9 \times 10^{-4} \text{ mol dm}^{-3}$ . (Patil *et al.* note that these experiments were performed at lower concentrations of methylene blue for which additional aggregation, such as trimerization, did not occur. Such would be expected at higher concentrations in an optical medium, and this process would further red-shift the spectrum).

Example 1, disclosed by Applicants at paragraph [0063] in the prepublication, shows an operable invention created by dissolving 250 mg of MB-3, [(7(dipropylamino) phenothiazine-3-ylidene) dipropyl-amine], in a 25 ml volume to create a 10 g/L solution of MB-3. The formula weight of MB-3 is  $447.9 \text{ g mol}^{-1}$ . The resultant total molar concentration, before drying and concentrating on an optical disc, was  $2.2 \times 10^{-2} \text{ mol dm}^{-3}$ .

Using even this low concentration, which must greatly increase after solvent is removed while making the invention, Applicants calculate that a hypothetical methylene blue solution of this same concentration in water, as was the case in Karasawa *et al.*, would be predominantly in the dimer state. Using Equation (1) from the Patil reference, Applicants calculate that the percentage of dye molecules in the monomer form,  $\alpha$ , would be only 7.8%, with  $K = 2.9 \times 10^{-4} \text{ mol dm}^{-3}$  as measured by Patil *et al.* (see Appendix B). Examiner will recognize thereby that 92.2% of the molecules would be in the dimer form. Examiner will also recognize that as such an aqueous film dries, as in the Karasawa invention, the equilibrium will shift further to the dimeric state by mass action, and that the fraction of

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-16-of-27-

monomeric methylene blue molecules would decrease further still. Applicants thereby conclude that at most a few percent, if any, of the methylene blue molecules would be in the monomeric state in the invention described by Karasawa *et al.* as applied to the current case.

Further, from Fig. 3 in Patil *et al.*, it is seen that the molar extinction coefficient of the methylene blue monomer is about  $4.8 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 650 nm. The corresponding molar extinction coefficient of the dimer aggregate is about  $0.4 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 650 nm.

Thus, the extinction coefficient of the monomer is about 12x that of the dimer at 650 nm. Example 1, of the instant application, demonstrates an optical density of 0.22 at 650 nm, using MB-3, the bis-propyl compound of the invention that was synthesized to minimize dimerization. Hypothetically assuming, instead, that methylene blue were used in place of MB-3 under the same conditions (as in Karasawa *et al.*), and that the monomers have roughly the same extinction coefficients, the resultant mixture of dimer at the same concentration would yield an absorbance (A) of roughly  $0.22/12 = 0.018$ , rather than 0.22. Using Lambert's Law for absorbed light,  $(I - I_0)/I_0 = 1 - 10^{-A}$ , methylene blue dimer would absorb roughly 10% of the incident light, while MB-3 would absorb roughly 50% of the incident light, a five-fold advantage.

From this reasonable hypothetical example, based on independent experimental evidence provided by Patil *et al.*, the Examiner will see that methylene blue, as Applicants have equitably stated and directly observed, is inoperable for the instant invention because of the phenomenon of aggregation. The phenomenon of aggregation, manifested in a condensed phase such as the coating of an optical disc, and dependent on molecular steric factors, unpredictably and greatly modifies the light absorption characteristics of a simple thiazine-type dye from that obtained in dilute solution. Thus, as Applicants have found, such spectra are not predictive of the operability of the invention.

Applicants have provided authoritative, independent evidence for their arguments that methylene blue behaves fundamentally and unexpectedly differently than propylene blue. The phenomenon of aggregation alters the absorption spectrum of methylene blue such that the spectrum of the aggregate form is greatly shifted from that of propylene blue.

Finally, Applicants have now amended claim 22 to specifically negatively limit methylene blue from the claim. Applicants stress that the claim has previously implicitly

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-17-of-27-

negatively limited the use of methylene blue, because the claim has read: "An optical disc comprising a composition having: an electron transfer agent; and *a dye activatable by a wavelength of between about 630 nm to about 660 nm* to convert from one optical state to a second optical state..."(emphasis added). Applicants had previously directly demonstrated this fact in one example.

In summary, claim 22 requires that the dye be activatable by a wavelength of between about 630 nm to about 660 nm on the optical disc when in an optical disc coating. As stated at paragraph [0015] of the specification, it was unexpected to the inventors that a lambda shift may occur when dye substances are placed into the coating of an optical medium in concentrated form. As evidenced, optical spectra obtained in dilute solution, are not predictive of the effect. Given the difficulties involved in identifying such shift, which depends on the ability of a compound to aggregate, the need to do so by experimentation, the fact that methylene blue was found in actual practice not to be useful at such wavelengths, Applicants aver that the sterically unhindered compounds in the Karasawa *et al.* JP 55-079441 reference can not teach, suggest or imply the embodiments asserted in claim 22, or claims 23 or 24 that depend therefrom.

As indicated above, the reference cited by the Examiner does not anticipate the claims, and lacks the claimed embodiments. Applicants therefore respectfully request that such rejections be withdrawn.

- Rejections under 35 U.S.C. 103(a)

*Rejections of claims 22-24 under 35 U.S.C. 103(a) as being unpatentable over Karasawa et al. JP 55-079441*

- Examiners Position

At paragraph 5, page 4 of the office action Examiner rejects claims 22-24 under 35 U.S.C. 103(a) as being unpatentable over Karasawa *et al.* JP 55-079441. According to the Examiner, with respect to the embodiments not anticipated, it would have been obvious to one skilled in the art to modify the cited example by using the other disclosed amines, including the polymeric amines disclosed on page 3, with a reasonable expectation of

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-18-of-27-

forming a useful optical recording medium. Examiner argues that the methylene blue based compositions and changes suggested by the reference would be embraced by claims 22-24.

- Applicants Response

Applicants herein address the Examiner's 103(a) rejections of claims 22-24 as set forth in the Examiner's Office Action. Applicants respectfully traverse the rejections of claims 22 – 24 based in part on the argument that, as amended, simple methylene blue based compositions are now specifically excluded by claim 22, and also that the combining references do not teach, suggest, or motivate combination of elements to produce the instant invention.

Above, applicants have credible, reasonable independent evidence that methylene blue, concentrated in a film on a substrate, such as in the case of the Karasawa reference, changes spectrum drastically. The concentrated form does not in fact resemble its' spectrum in dilute solution, wherein the monomer form predominantly exists. The phenomenon is most likely caused by aggregation, and produced an unexpected result when such a dye was placed in a polymer film such as on a an optical disc. Also, Applicants have directly shown that methylene blue was an inoperable dye in the instant invention, and that experimentation was needed to find dyes that were operable.

Regardless of the other disclosed amines, the failure of methylene blue to produce an operable invention, as an example by direct experimentation, teaches away from using dyes of its class to accomplish the goals of the invention.

Also, as discussed above, Applicants make clear that methylene blue was implicitly excluded from claim 22. However, to further clarify, Applicants have added a negative limitation to claim 22, specifically excluding methylene blue.

Based on the arguments presented above, Applicants respectfully request that such rejections of claims 22-24 be withdrawn.

*Rejections of claims 4-14 and 22-24 under 35 U.S.C. 103(a) as being unpatentable over Karasawa et al. JP 55-079441, in view of Taylor*

- Examiner's Position

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-19-of-27-

At paragraph 6, page 5, the Examiner rejects claims 4-14 and 22-24 under 35 U.S.C. 103(a) as being unpatentable over Karasawa *et al.* JP 55-079441, in view of Taylor (J. Histochemistry and Cytochemistry, vol. 8, pp. 248-257 (1960)). At the end of paragraph 6, at page 6, Examiner makes emphatically clear that the rejection of claims in this section does not apply to the composition applied to the ROM medium. Examiner states that the Taylor reference teaches tetrapropyl, diethyl/dimethyl, tetraethyl dyes in table I on page 256 and includes their absorption maxima. As stated by the Examiner, Figure 7 evidences such spectra being more than 150 nm wide and centered about 610-650 nm. Examiner states that it would have been obvious to use other alkyl groups, such as propyl or ethyl, in place of at least one of the N-methyl groups as disclosed in Taylor in place of the methyl groups used in the example of Karasawa *et al.* Examiner disagrees that the references are non-analogous, noting that in both cases the references are concerned with the absorption of dyes. As stated by the Examiner, "Removing the "transient" language does not preclude transient reactions." It broadens the claims. Finally, the Examiner argues that the spectroscopic data and similarity of the compounds leads one to make the substitution with a reasonable chance of success.

- Applicants Position

Applicants herein address the Examiner's 103(a) rejections of claims 4-14 and 22-24 as set forth in the Examiner's Office Action. Applicants respectfully traverse the rejections of the claims based in part on the argument that the combining references do not teach, suggest, or motivate combination of elements to produce the instant invention, and that the aggregation behavior of the simple thiazine dyes such as methylene blue in concentrated form was unpredictable from solution spectra.

Applicants note that claims 22-24 are directed to a composition on an optical disc such as a ROM. Thus Examiner's statements that the rejections under this heading do not apply to the composition of the invention applied to a ROM seem contradictory, and Applicants respectfully seek clarification.

With respect to the two references, the gist of Examiner's argument is summarized at lines 3-5 on page 6 of the Office Action: " The spectroscopic data and the similarity of the compounds leads one to make the substitutetion[sic] and have a reasonable expectation i[n]

CT01/WINTJ/249874.1



Atty. Ref.: VT1-114.8B(CIP)

-20-of-27-

success of forming a useful optical recocirding[sic] medium with performance similar to that of Karasawa et al. JP 55-079441."

Applicants have pointed out, as above, that absorption spectra obtained under the conditions of the Taylor reference are not representative of the spectra obtained in more concentrated forms as may be required to construct an operable invention such as a optical state change security composition affixed to an optical recording medium. For instance, as pointed out in the Patil *et al.* reference (Appendix A), the absorption spectrum of methylene blue shows very significant modification and red-shifting of intensity, even at a concentration of  $4 \times 10^{-4}$  M (see Fig. 1, page 4314, spectrum "D"). Simple calculation (Appendix B) shows that at a modest concentration of  $2.2 \times 10^{-2}$  M, over 90% of the dye would be in a dimeric state that absorbs very poorly at 650 nm, the read wavelength of a majority of standard DVD readers. Applicants have also demonstrated in fact that a disc comprising a polymer film including methylene blue does not function in the operable manner of the invention.

Because of the phenomenon of aggregation, such as dimerization, the behavior of simple thiazine type dyes upon concentration is not predictable from the spectra such as those found in the Taylor reference. This is because the phenomenon is dependent on steric factors that are not revealed by an absorption spectrum.

Further elaboration is needed to describe the state of the photochemical arts, currently and prior to the time of filing of the instant application. It was, and is well known that any photochemical process may have a broad variety of outcomes, depending on the delicate balance of rate factors for self-reaction, emission, decomposition or relaxation of an excited molecule, and that these factors are much more complicated in the case of further reactions with other molecules, as is the case here. Because of these competing rate factors, photochemical reactions rarely follow any form of structure activity correlation. The Examiner can see this even with the limited behavior evidenced here. For instance, changing the thiazine dye substitution from methyl to propyl, (from methylene blue to propylene blue) leads to completely different behaviors. In the first case, the molecule aggregates and the aggregate is the photochemically active species. In the second case, the monomer principally obtains, and the monomer is the photochemically active species. The photophysical properties of the dimer form are greatly different than the monomer form, and as a

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-21-of-27-

consequence it is not possible to reliably predict system behavior in advance of experimentation.

Applicants can find no teaching, suggestion, or motivation therein to combine the disparate elements disclosed in Karasawa *et al.* and Taylor. Further, if there were, based on common knowledge at the time, any such combination would have been recognized to be unpredictable. Given such knowledge, broad and specific, there would not be a reasonable expectation of success. In view of the considerations, Applicants therefore respectfully request that such the rejections of claims 4-14 and 22-24 be withdrawn.

*Claim 1, 14-15, and 21 Rejections Under U.S.C. 35 103(a) as being Unpatentable over Smith et al. in View of Taylor*

- Examiner's Position

Claims 1, 14-15, and 21 stand rejected Under U.S.C. 35 103(a) as being unpatentable over Smith *et al.* (U.S. 5,815,484) in View of Taylor (J. Histochemistry and Cytochemistry, vol. 8, pp. 248-257 (1960)) (see paragraph 7, page 6 of the Office Action) According to the Examiner, Smith *et al.* teach the use of various phenothiazines including methylene blue and Toluidine blue in copy prevention of optical media. Further, as stated by the Examiner, it would have been obvious to use other alkyl group substitution as disclosed by Taylor. The Examiner indicates that the question is one of spectroscopic equivalence of the dyes, and suggests, at lines 5-7, page 7 of the Office Action, that Applicants might provide data evidencing unexpected results when propylene blue is used in place of methylene blue.

- Applicant's Position

Applicants herein address the Examiner's 103(a) rejections of claims 1, 14-15 and 21 as set forth in the Examiner's Office Action. Applicants respectfully traverse the rejections of claims based in part on the argument that the combining references lack the necessary combining elements. Further, there is neither taught nor suggested a reason to combine, nor is motivation provided. Additionally, the behavior of methylene blue with respect to propylene blue yields unexpected results.

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-22--of-27-

Note that Applicants have negatively limited methylene blue from its amended claims by specific action. Further, Applicants again state that Smith *et al.* teach the use of leucomethylene blue, rather than methylene blue. The Examiner will readily see that if methylene blue were substituted for leucomethylene blue, the invention of Smith *et al.* could not function, because the invention requires an initial transparent state. Smith *et al.* teaches against the use of methylene blue as an initial composition, and provides the element of air oxidation for disabling the read mechanism of a compact disc. There is no element of photochemical activation of the methylene blue.

With respect to claim 1, no element of photochemical activation of methylene blue is found in Smith *et al.*, as is true for the Taylor reference also. Thus, Examiner's rejections lack authority over claim 1, because the necessary elements can not be taught by the references in any aspect.

The absorption spectra disclosed by Taylor are of thiazine type dyes, not their *leuco* forms. Applicants can find no obvious reason to combine elements of the invention of Smith *et al.*, which directs toward the use of uncolored *leuco* dye forms, with the spectra of Taylor that comprise only the highly colored native forms.

Regarding the Examiners invitation to provide evidence of unexpected results, Applicants provide the following data of record evidencing unexpected results when propylene blue is used in place of methylene blue;

Methylene blue has a spectral absorption maximum near 655 nm in solution (US 2004/0152017, [0060]), whereas propylene blue has an absorption maximum near 650 nm (US 2004/0152017, [0063]). The dyes absorb light similarly in solution. However, when placed on an optical disc in concentrated form, methylene blue has an absorption maximum near 590 nm (US 2004/0152017, [0060]) and absorbs 650 nm light poorly. In contrast, under similar conditions, propylene blue has an absorption maximum "in the expected range" and absorbs substantially at 650 nm (US 2004/0152017, [0064]). The discovery of the unexpected behavior of methylene blue is also reported at paragraph [0017](US 2004/0152017).

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-23-of-27-

The reasons for this behavior have been rationalized, as above. In concentrated form, methylene blue dimerizes (Patil *et al.*) while propylene blue possesses the proper steric factors to minimize dimerization, a process that requires close molecular contact between partners. Examiner will realize that steric factors are not predictable from absorption spectra.

Applicants can find no teaching, suggestion, or motivation therein to combine the disparate elements disclosed in Smith *et al.* and Taylor. Further, the necessary combining elements are not present. Taylor references colored dye forms. The invention of Smith *et al.* would be inoperable from the start if such forms were used. Finally, the substitution of propyl for methyl on the thiazine dye structure yields results that could not be predicted from the spectra of Taylor, and certainly not from Smith *et al.*

In view of the above considerations, Applicants respectfully request that such the rejections of claims 1, 14-15 and 21 be withdrawn.

*Claim 1, 14-15, and 21 Rejections Under U.S.C. 35 103(a) as being Unpatentable over Selinfreund et al. in view of Smith et al. and Taylor.*

- Examiner's Position

Claims 1, 14-15, and 21 stand rejected Under U.S.C. 35 103(a) as being unpatentable over Selinfreund *et al.* (WO 02/03386) in view of Smith *et al.* and Taylor (J. Histochemistry and Cytochemistry, vol. 8, pp. 248-257 (1960)) (see paragraph 8, page 7 of the Office Action) According to the Examiner Selinfreund *et al.*, especially at Figure 8, teach a variety of relevant elements including an optical recording medium wherein the light sensitive materials are provided over the whole medium, or on one surface at predetermined areas. The Examiner further states that an embodiment of a security dye coated on a CD is disclosed, as are examples that describe a medium with authentication software. The Examiner further states that the use of methylene blue with DVD media is disclosed, as are other embodiments of optical media construction. According to the Examiner, it would be obvious to one skilled in the art to modify the embodiment of figure 8 by using the teachings of Smith *et al.* and the disclosure of Taylor whereby different alkyl groups such as ethyl or propyl are substituted for the methyl groups of methylene blue used by Smith *et al.*, and Selinfreund *et al.* with a reasonable expectation of success.

CT01/WDNTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-24-of-27-

- Applicant's Position

Applicants herein address the Examiner's 103(a) rejections of claims 1, 14-15 and 21 as set forth in the Examiner's Office Action. Applicants respectfully traverse the rejections of claims based in part on the argument that the combining references lack the necessary combining elements.

The absorption spectra disclosed by Taylor are of thiazine type dyes, not their *leuco* forms. Applicants can find no obvious reason to combine elements of the invention of Smith *et al.*, which directs toward the use of uncolored *leuco* dye forms, with the spectra of Taylor that comprise highly colored native forms. Certainly no teaching, suggestion, or motivation is provided in the references.

Selinfreund *et al.*, in table 1, page 21, indicate that methylene blue may be (*i.e.*, possibly) be used as a security element, but the suggestion was prophetic, and later shown to be inoperable, as has been repeatedly stated in this Amendment.

In view of the above considerations, in part that the necessary elements are not present in the combining references, and that methylene blue has been specifically declaimed from the instant application, Applicants respectfully request that such the rejections of claims 1, 14-15 and 21 be withdrawn.

*Claim 1, 14-15, and 21 Rejections Under U.S.C. 35 103(a) as being Unpatentable over Selinfreund et al. in view of Smith et al. and Taylor.*

- Examiner's Position

Claims 1, 14-15, and 21 stand rejected Under U.S.C. 35 103(a) as being unpatentable over Selinfreund *et al.* ('631) in view of Taylor (J. Histochemistry and Cytochemistry, vol. 8, pp. 248-257 (1960)) (see paragraph 8, page 7 of the Office Action) According to the Examiner Selinfreund *et al.* teaches in claims 36-52 the optical recording medium where the light sensitive materials which affect authentication are provided over the whole medium which is then coated with a reflective layer. The Examiner indicates that [0053] describes a medium provided with authentication software and the use of methylene blue with DVD media is disclosed in table 1, page 7. Examiner states that it would have been obvious to

CT01/WNTJ/249874.1

Atty. Ref.: VTJ-114.8B(CIP)

-25-of-27-

modify the embodiment of claims 36-52 in Selinfreund *et al.* '631 by using other alkyl groups, as disclosed in the Taylor reference in place of at least one methyl groups of the methylene blue as used by Selinfreund.

- Applicant's Position

Applicants herein address the Examiner's 103(a) rejections of claims 1, 14-15 and 21 as set forth in the Examiner's Office Action. Applicants respectfully traverse the rejections of claims based in part on the argument that the art described therein is of such unpredictability that one skilled in the art would not find the combined teaching to make obvious the instant invention as in claims 1, 14-15 and 21.

Applicants have discussed at length herein the difficulty of discovering a dye useful for the invention, and that small structural differences in the dye structure result in large differences in behavior of the dye. As has been noted, both methylene blue and propylene have similar absorption spectra in solution, are structurally similar, but they behave quite differently in concentrated form, as may be necessary to form an operable method or optical medium as in claims 1, 14-15 and 21. The phenomenon is apparently related to aggregation, and this process is not predictable from solution spectra, such as those disclosed by the Taylor reference. Hence, one skilled in the art would not find obvious the invention of the subject claims when considering the two references cited by the Examiner. Taylor lacks the predictability needed to rise to the point of obviousness when combined with the Selinfreund reference.

In view of the above considerations, in part because of unpredictability in the art, Applicants respectfully request that such the rejections of claims 1, 14-15 and 21 be withdrawn.

- Rejection under Judicially Created Doctrine of Obviousness-type Double Patenting

- Examiner's Position

The Examiner has rejected all of the claims, claims 1 and 4-24, under the judicially created doctrine of obvious-type double patenting as being unpatentable over claims 1 – 17 of U.S. Patent No. 6,952,392. The Examiner argues that although the conflicting claims are not

CT01/WINTJ/249874.1

Atty. Ref.: VTI-114.8B(CIP)

-26-of-27-

identical, it would be obvious to use the propylene blue compounds based upon the direction in claim 8 to the substituents being propyl or hexyl.

- Applicants' Response

Applicants respectfully request that the Examiner hold this rejection in abeyance until patentable claims are to be issued in this application. Applicant if necessary will file an appropriate terminal disclaimer.

- Prior Art Made of Record but Not Relied Upon

- Examiner's Position

Examiner has made no new art of record.

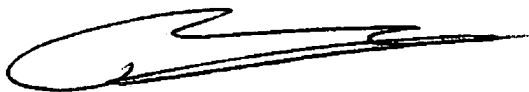
- Applicants Response

No response is needed by Applicants.

CONCLUSION TO REMARKS

Applicants assert that this response is fully responsive to the Examiner's Office action dated December 6, 2006. Applicants respectfully seek early allowance of the pending claims.

Respectfully Submitted,



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Atty. Ref.: VTI-114.8B(CIP)

-27-of-27-

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## VI. APPENDIX

- A. "Self-aggregation of Methylene Blue in aqueous medium and aqueous solutions of Bu<sub>4</sub>NBr and Urea" by Patil *et al.*,
- B. Calculation of the Fraction ( $\alpha$ ) of Methylene Blue in Monomer Form in an Exemplary case



## Self-aggregation of Methylene Blue in aqueous medium and aqueous solutions of Bu<sub>4</sub>NBr and urea

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The self-aggregation of Methylene Blue [MB] ( $1 \times 10^{-6}$ – $4 \times 10^{-4}$  M) in water and in aqueous solutions of Bu<sub>4</sub>NBr (0.1–0.3 M) and urea (0.1–4 M) has been investigated by recording electronic spectra in the wavelength range 550 to 700 nm. Analysis of the spectral data yielded the dimer dissociation constant, and individual characteristic monomer and dimer spectra. By applying the exciton model for the first time, the interaction energy between the MB molecules in the dimer species has been evaluated. Further, information about the dimer geometry and the twist angle between the dipoles of the MB molecules in the dimer species has been obtained. Similar data have also been obtained for solutions of MB in aqueous urea and Bu<sub>4</sub>NBr solutions. The significant observation is the change in the dimer geometry from sandwich type to end-on-end type in the presence of urea and Bu<sub>4</sub>NBr. It is observed that the contribution to the interaction energy is mainly from van der Waal's type and dispersion forces, in addition to short-range forces involving multipoles. However, the contribution from hydrogen-bonding interactions between MB and water molecules is found to be significantly low. It is proposed that water structural effects and hydrophobic interactions are the major factors in the phenomenon of aggregation.

### 1 Introduction

The phenomenon of aggregation of dyes, drugs, surfactants, etc., has been extensively studied by *e.g.* spectrophotometry, light scattering, osmometry, polarography, etc.<sup>1,2</sup> The results of these studies have indicated that the dye molecules, in spite of repulsion due to similar charges (cationic dyes), undergo self-aggregation to form dimer, trimer and higher aggregates,<sup>3</sup> the charges being maintained as far as possible in the aggregate.<sup>4</sup> The forces which hold the dye molecules together are believed to be hydrogen bonding, van der Waal's forces, London dispersion forces and other short-range forces.<sup>1</sup> The most predominant force has been recognized to be hydrophobic interactions, which help the system to get rid of the thermodynamically unfavourable state (of negative  $\Delta S$  due to hydrophobic hydration). Thus the water structural features seem to play the role of catalyst in inducing aggregation.

The state of a thiazine dye, MB in water has been investigated by several workers.<sup>5–8</sup> The studies of Mukerjee *et al.* have shed light on the intermolecular interactions involved in the formation of the dimer and trimer of MB.<sup>8</sup> The inferences drawn by them can be examined by application of the exciton model, proposed by Kasha *et al.*,<sup>9</sup> with the help of detailed analysis of the spectral data of MB in aqueous salt and aqueous non-electrolyte solutions. Thus, we have carried out systematic spectrophotometric studies of the state of MB in aqueous salt solutions and aqueous urea solutions in the concentration range of the dye where aggregation does not exceed dimerization.

### 2 Experimental

The spectra were recorded using an Elico SL159 UV-VIS Spectrophotometer in the wavelength range 550 to 700 nm. The matched quartz cuvettes of pathlengths 1, 0.5 cm and 0.1 cm were used for the purpose of recording the spectra such

that the absorbance values do not exceed 1.5. The pathlengths of the cuvettes were found to match within  $\pm 0.001$  cm. All the measurements reported in the present work were carried out at  $27 \pm 1^\circ\text{C}$ .

The purity of MB (hydrochloride) procured from Qualigens Fine Chemicals was assessed by recording the spectrum of its solution in absolute alcohol (GR, E. Merck). The  $\lambda_{\text{max}}$  value obtained was  $656 \pm 1$  nm, while the molar absorption coefficient was found to be  $6.8 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ . The spectrum of MB in water ( $1 \times 10^{-6}$  M) has a shape similar to that reported in the literature with  $\lambda_{\text{max}} = 664 \pm 1$  nm.<sup>5–8</sup> Hence, the dye was used without further purification.

Tetra-butyl ammonium bromide (John Baker Inc., Colorado, USA) was dried at  $50$ – $60^\circ\text{C}$  in a vacuum oven, while urea procured from Qualigens Fine Chemicals was stored in an evacuated desiccator. All solutions were prepared on a molarity basis. The stock solution of MB, stored in a reagent bottle wrapped with black paper, was used within 1 week of its preparation.

### 3 Results

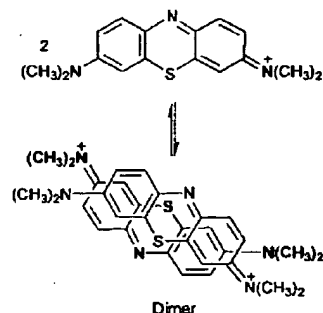
The visible spectra of MB in water, in the concentration range  $1 \times 10^{-6}$  to  $4 \times 10^{-4}$  M (7–8 concentrations), were recorded at  $27 \pm 1^\circ\text{C}$ . The spectra at four concentrations are reproduced in Fig. 1. Examination of Fig. 1 reveals that with increasing concentration the peak at 605 nm, due to the dimer, increases in intensity at the expense of the intensity of that at 664 nm, attributed to the monomer. An approximate isosbestic point was observed at 630 nm. The data obtained were analyzed using the following methodology:

Since the concentration of MB was maintained below the concentration at which appreciable trimerization begins, the only equilibrium believed to be existing in the solutions is the monomer–dimer equilibrium,<sup>7</sup>

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The dimer dissociation constant ( $K$ ) can be expressed as:

$$K = \frac{C_M^2}{C_D} = \frac{2\alpha^2 C}{(1-\alpha)} \quad (1)$$

where the total dye concentration,  $C = C_M + 2C_D$ ,  $C_M$  and  $C_D$  being the concentrations of MB in the monomeric and dimeric forms respectively and  $\alpha$ , the fraction of the dye molecules existing in monomeric form. Further the molar absorption coefficient ( $\epsilon$ ) of MB can be expressed as a function of those of the monomer ( $\epsilon_M$ ) and dimer ( $\epsilon_D/2$ ) species, assuming that the Beer-Lambert law is applicable to both the species.

$$\epsilon = \alpha\epsilon_M + (1-\alpha)\epsilon_D/2 \quad (2)$$

Using the boundary conditions,  $\epsilon_D/2 = \epsilon$  when  $\alpha = 0$  and  $\epsilon_M = \epsilon$  when  $\alpha = 1$ , and applying a least-squares fit (Fig. 2) to a rearranged form of eqn. (2),  $\epsilon_M$  and  $\epsilon_D/2$  were evaluated. The value of  $K$  was accepted only if the regression coefficient of the above fit was above 98%. These calculations were repeated for at least 10–15 wavelengths to verify that the value of  $K$  obtained was independent of wavelength and hence close to the real value. The  $\epsilon_M$  and  $\epsilon_D/2$  values obtained at different wavelengths allow us to generate the monomer and the dimer

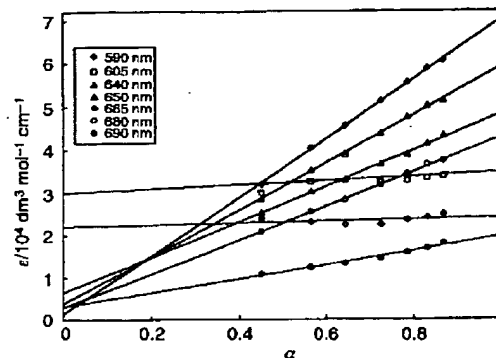


Fig. 2 Molar absorption coefficient vs. degree of association at different wavelengths.

spectra (Fig. 3). Further, the concentration of the monomer and dimer species were calculated with the help of  $\epsilon$  and  $\alpha$  values. It was observed that the plot of  $\log C_D$  against  $\log C_M$  (eqn. (1)) results in a straight line with a slope of 2.3, as compared to the expected value of 2.0. This substantiates our assumption that the monomer-dimer equilibrium is the only predominant equilibrium existing in the solution under investigation. Further, both the monomer and the dimer species were found to follow the Beer-Lambert law individually at the respective wavelengths, giving credence to the use of eqn. (2). Thus the value of  $K$  so obtained can be accepted with confidence.

The most suitable value for the dimer dissociation constant was found to be  $2.9 \times 10^{-4} \text{ mol dm}^{-3}$ . This is in good agreement with the value  $2.8 \times 10^{-4} \text{ mol dm}^{-3}$  reported by Rabinowitch and Epstein<sup>5</sup> from their work carried out at 26.7°C, while it is comparatively higher than the  $1.7 \times 10^{-4} \text{ mol dm}^{-3}$  obtained by Bergman and O'Kinski<sup>6</sup> at 25°C (their solutions were buffered to pH 4.75). The value of  $K$  is smaller than the values reported by Mukerjee *et al.*,<sup>8</sup>  $4.4 \times 10^{-4} \text{ mol dm}^{-3}$  (25°C) and Braswell<sup>7</sup>  $5 \times 10^{-4} \text{ mol dm}^{-3}$  (30°C).

The oscillator strengths ( $f$ ) of the monomer and the dimer species were evaluated using:<sup>10</sup>

$$f = 1.44 \times 10^{-19} A \quad (3)$$

where  $A = \int \epsilon(\nu) d\nu$ , i.e., the area under the curves while the corresponding transition dipole moments ( $\mu$ ) were calculated

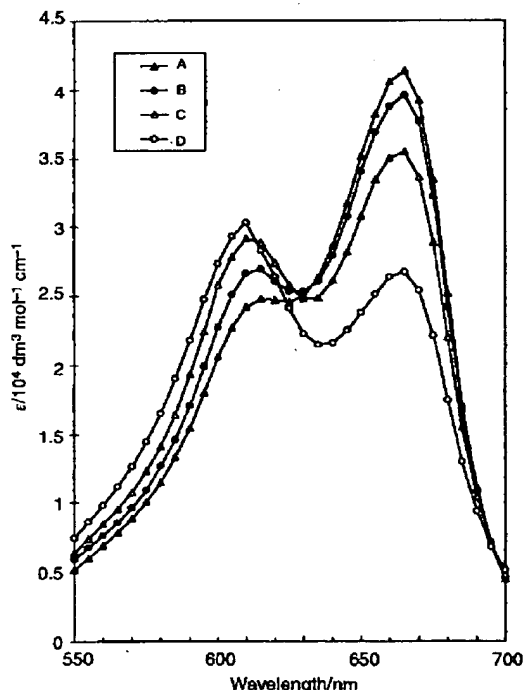


Fig. 1 Spectra of MB in water [concentrations (A)  $5 \times 10^{-5} \text{ M}$ ; (B)  $1 \times 10^{-4} \text{ M}$ ; (C)  $2 \times 10^{-4} \text{ M}$ ; (D)  $4 \times 10^{-4} \text{ M}$ ] at 27°C.

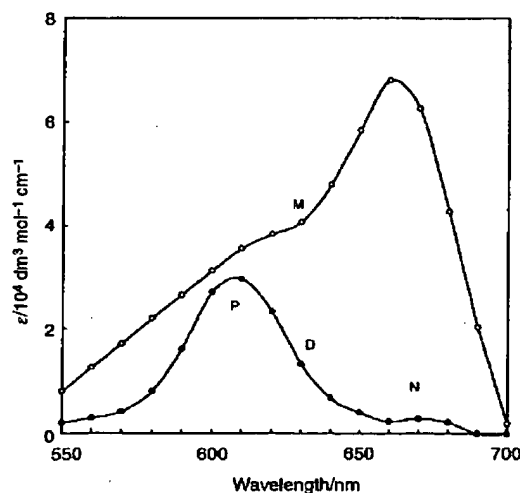


Fig. 3 Resolved monomer and dimer spectra of MB in water.

**Table 1** The dimer dissociation constant and the spectral data of MB in aqueous and in aqueous Bu<sub>4</sub>NBr and urea solutions at 27 °C

Solvent	$K/10^{-4}$ mol dm <sup>-3</sup>	$\epsilon_d/10^4$ dm <sup>3</sup> mol <sup>-1</sup> cm <sup>-1</sup>	$\epsilon_d/2 \times 10^4$ dm <sup>3</sup> mol <sup>-1</sup> cm <sup>-1</sup>		$\epsilon_N/\epsilon_P$	$\theta/$ degrees	$f$		$\mu/10^{-30}$ C m		$U/$ cm <sup>-1</sup>
			P	N			M	D	M	D	
water	2.9	6.8	3.0	0.3	0.1	31.6	0.27	0.07	11.8	5.9	801.5
Bu <sub>4</sub> NBr											
0.1 M	0.3	6.8	1.4	1.9	1.4	100	0.40	0.09	14.4	6.5	621.0
0.2 M	0.6	5.9	1.5	2.2	1.5	102	0.24	0.10	11.1	6.9	611.3
0.3 M	1.5	5.7	1.1	1.4	1.3	98	0.23	0.07	10.9	5.8	546.0
Urea											
0.1 M	0.7	5.5	1.3	2.2	1.7	105	0.27	0.06	11.8	5.2	815.0

using:<sup>10</sup>

$$f = \frac{8\pi^2 m \nu |\mu|^2}{e^2 3h} \quad (4)$$

where  $m$  and  $e$  are the mass of and charge on the electron,  $\mu$  the transition dipole moment,  $h$  Planck's constant and  $\nu$  the frequency at maximum absorbance. These parameters for MB in water are collected in Table 1.

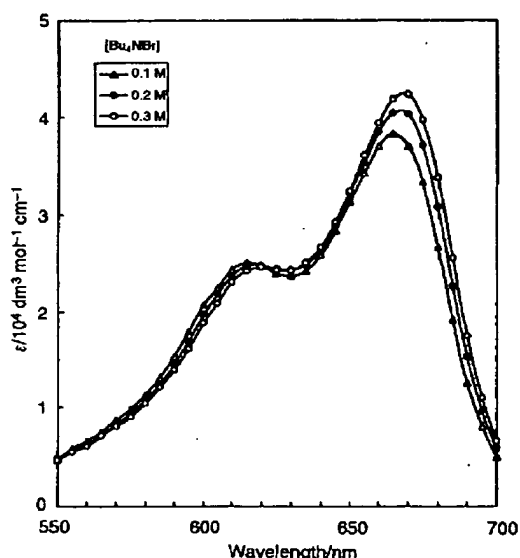
Application of the exciton model<sup>9</sup> to the dimer species reveals that the ground state of the dimer remains doubly degenerate, while the excited state splits into two different energy levels. The difference in energy of the split levels,  $\Delta E$ , depends on the interaction ( $U = \Delta E/2$  cm<sup>-1</sup>) between the dye molecules in the dimer species.<sup>2,11</sup> For a perfectly sandwich type dimer the transition from the ground state to the higher excited state is allowed, resulting in the P-branch, which appears on the shorter wavelength side of the monomer band. In the case of the end-on-end type of dimer the transition to the lower excited state is the only allowed transition, resulting in a band on the longer wavelength side of the monomer band, called the N-branch (Fig. 3). Since the real systems are non-ideal, we always find some intensity for both bands. The ratio  $\epsilon_N/\epsilon_P$ , the molar absorption coefficients of the dimer species at the  $\lambda_{max}$  of the N and P-branches respectively, gives some indication of the dimer geometry.<sup>2</sup> Our results (Table 1) indicate that the MB dimer in water exists almost entirely as a

sandwich-type dimer. Further, the angle between the transition dipoles of the MB molecules in the dimer can be evaluated using<sup>2</sup>

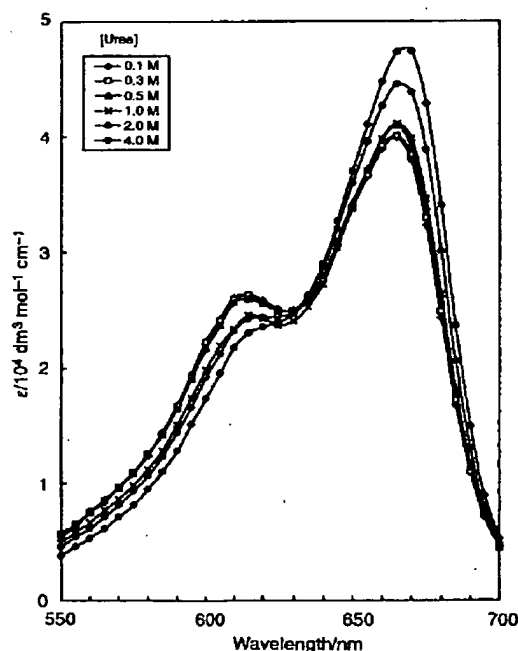
$$\tan^2 \theta/2 = \epsilon_N/\epsilon_P \quad (5)$$

assuming that the distance between the dipoles remains unchanged.

Similar data for MB in aqueous Bu<sub>4</sub>NBr and aqueous urea solutions are reproduced in Fig. 4 and 5, respectively. It is observed that both the solutes decrease the association of the dye molecules. Fig. 6 and 7 show the monomer and dimer spectra of MB (at one representative concentration of the additive) in the presence of Bu<sub>4</sub>NBr and urea, respectively. The dimer spectrum in the presence of Bu<sub>4</sub>NBr and urea as compared with that in water, shows enhancement of the intensity of the N-branch relative to the P-branch. A similar dimer spectrum has been observed in the presence of KI, whereas in presence of KBr both P and N-branches have equal intensity.<sup>12</sup> The dimer dissociation constant, oscillator strength, transition dipole moment and the twist angle ( $\theta$ ) for these systems are collected in Table 1.



**Fig. 4** Spectra of MB in aqueous Bu<sub>4</sub>NBr solutions at 27 °C. [MB] =  $1 \times 10^{-4}$  M.



**Fig. 5** Spectra of MB in aqueous urea solutions at 27 °C. [MB] =  $1 \times 10^{-4}$  M.

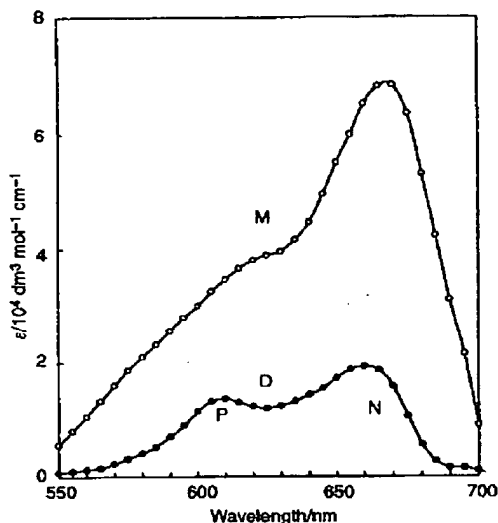


Fig. 6 Spectra of the monomer and dimer species of MB in aqueous solutions of  $\text{Bu}_4\text{NBr}$ .  $[\text{Bu}_4\text{NBr}] = 0.1 \text{ M}$ .

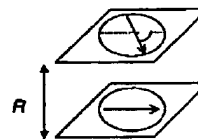
#### 4 Discussion

The spectral data presented in Fig. 1 clearly indicate that with increase in concentration of MB the extent of aggregation also increases. The role played by water structural effects (entropy and enthalpy contributions) in aqueous dye solutions has been discussed earlier by Mukerjee *et al.*<sup>8</sup> They concluded that dispersion forces are the important contributors to interaction forces, in addition to the driving force provided by the water structural effects. Application of the exciton model<sup>9</sup> has provided sufficient information about the dimer geometry to allow qualitative extension of their interpretations.

The interaction energy in the dimer can be evaluated by application of the exciton model, as discussed earlier. In the case of the dimer of MB in water, we obtained a value of 801.8

$\text{cm}^{-1}$  ( $9.6 \text{ kJ mol}^{-1}$ ), which is definitely less than if H-bonding were the dominant factor ( $\sim 20 \text{ kJ mol}^{-1}$ ). This is in accordance with simulation studies of the hydration of benzene in dilute aqueous solutions, which indicate that the hydrogen-bonding interaction between water molecules and the  $\pi$ -electron system of benzene is not appreciable.<sup>13</sup> However, the quadrupole moment of the benzene molecule does influence the orientation of the water molecules above and below the molecular plane. The interaction between the MB molecules in the dimer species should therefore, include interactions such as ion-dipole, dipole-dipole, dispersion forces and interactions involving higher multipoles. Of course repulsive interactions between similarly charged dye molecules would exist, but they seem to be dominated by the attractive cation-cation interactions. The charge-carrying dimethylamino groups on the two dye cations are so aligned as to achieve maximum charge separation.

The observed  $\epsilon_M/\epsilon_P$  ( $=0.1$ ) ratio in water is very small indicating, according to the exciton model, that the dimer geometry is of the sandwich type with a slight deviation from an exact parallel stacking arrangement of the dye molecular planes. Further the transition dipoles of the MB molecules in the dimer species are aligned, with respect to each other, through an angle,  $\theta = 31.6^\circ$  (Table 1). Thus the dipole-dipole and ion-dipole interactions will be of smaller magnitude than if the value of  $\theta$  were equal to zero. It is thus clear that the dispersion forces are contributing largely to the interaction energy due to greater mobility of the  $\pi$ -electrons.



The enhancement of the intensity of the 664 nm band (Fig. 4 and 5) at the expense of the intensity of the 605 nm band clearly indicates the decrease in tendency of the dye molecules to form aggregates.  $\text{Bu}_4\text{NBr}$  and urea are known to be efficient denaturants for proteins and macromolecules in water.<sup>14</sup> They cause increase in the solubility of hydrocarbons in water, however, the mode of action of the two solutes appears to be different. The hydrophobic hydration effects<sup>15</sup> in the aqueous solutions of  $\text{Bu}_4\text{NBr}$  (structure-making solute)<sup>16</sup> are similar to those exhibited by the monofunctional solutes like alcohols,<sup>17,18</sup> amines,<sup>19</sup> etc. Various experimental studies have indicated that the effect due to charge (of the  $\text{Bu}_4\text{N}^+$  ion) on water structural interactions is negligible in comparison to the hydrophobic effects but involves large cation-cation interactions.<sup>20</sup> When MB molecules are added to the structured aqueous environment, the hydration (hydrophobic) of these molecules is accompanied by a relatively small negative entropy change. Further, the formation of the dimer, therefore, will not lead to a positive entropy change as is observed in the case of the aqueous solutions of MB. The increase in dimer dissociation constant values (Table 1) with increase in concentration of  $\text{Bu}_4\text{NBr}$  also indicates the decreased tendency of the MB molecules to undergo aggregation. However, the values of the dimer dissociation constants at different concentrations of  $\text{Bu}_4\text{NBr}$  are less than those in an aqueous solution of MB. The reason may be sought in the existence of some new equilibria of the form of cation-cation ( $\text{MB-Bu}_4\text{N}^+$ ) attractive type interactions in addition to the monomer-dimer equilibrium. The bromide ion may act as a good counterion. We have not attempted to evaluate  $K$  taking into consideration these dye-ion binding type equilibria. This proposed hypothesis is supported on the basis of the  $(\epsilon_M/\epsilon_P)$  ratio, which is found to be about 1.5 (Fig. 6, Table 1) indicating, further, a greater probability of the transition to the lower

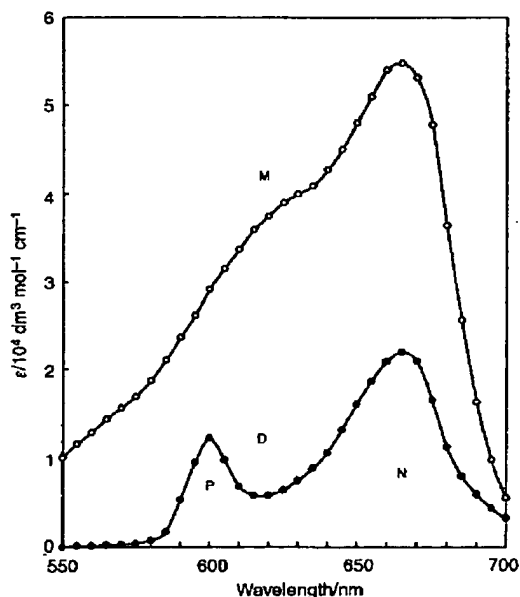


Fig. 7 Spectra of monomer and dimer species of MB in aqueous urea solutions.  $[\text{Urea}] = 0.1 \text{ M}$ .

excited state of the dimer species (exciton model). This in turn means that the dimer geometry has changed from sandwich type to the end-on-end type in aqueous Bu<sub>4</sub>NBr solutions. The proposed change in the type of dimeric species will thus be accompanied by exposure of greater surface area of the dye molecules in the dimer species to water, which as stated above is possible due to the relatively small negative  $\Delta S$  accompanying the hydration of the MB molecules in aqueous Bu<sub>4</sub>NBr solutions. The calculated value of the twist angle is about 100°. The van der Waal's as well as dispersion force interactions will be relatively reduced in magnitude, since close approach of the dye molecules, allowed in the case of the sandwich-type geometry, is now not possible. The interaction energies between MB molecules in the dimeric species in aqueous Bu<sub>4</sub>NBr solutions are found to decrease with increase in the Bu<sub>4</sub>NBr concentration (Table 1).

We now turn our attention to analysis of the spectral data for MB in aqueous urea solutions (0.1–4 M). The dimer dissociation constant of MB in the presence of urea could be satisfactorily evaluated only at 0.1 M concentration of urea. Further, the value of the dimer dissociation constant is smaller than that in aqueous solution of MB. This leads again to the conclusion that, in addition to the monomer-dimer equilibrium, there must exist some other equilibria involving urea-MB interactions (see below) in such solutions.

Urea is known to be a water-structure-breaking solute. According to Frank and Frank's model, urea causes increase in the fraction of dense water molecules (non-H-bonded water molecules).<sup>21</sup> It is also known that urea forms channel-like structures around hydrocarbon chains having chain length greater than 5 carbon atoms.<sup>22,23</sup> There are no reports of channel structures of urea around the aromatic moieties. Thus we may conclude that, in addition to water structural effects, there may exist certain specific urea-MB interactions, however, the exact nature of the interactions is hard to elucidate. We believe that the predominant interactions are mainly of van der Waal's and dispersion types. It is these interactions which switch the dimer geometry from the sandwich-type to the end-on-end-type ( $\epsilon_M/\epsilon_P = 1.7$ , Table 1). The present work alone cannot shed light on how the two different types of solutes, Bu<sub>4</sub>NBr (structure-making) and urea (structure-breaking), can cause a similar effect, i.e., reduction in the extent of aggregation of MB. The proposed MB-Bu<sub>4</sub>N<sup>+</sup>, MB-urea and [MB-Br] ion pair interactions can be better revealed if precise data in the dilute concentration region for the parameters like activity coefficients, conductance, etc. become available.

Finally, the oscillator strength for the monomer species of MB is found to be larger than that for the dimer form in all

three media studied (Table 1), indicating that the absorbing surface area per mole of the dimer species is reduced drastically. Furthermore, the transition dipole moment is also smaller for the dimer species than for the monomer in all the media studied. These results further indicate that the MB dimers are less efficient in trapping light. It is known that if the dimer is efficient as a trap for light, then a large reduction in fluorescence quantum efficiency is observed in the case of fluorescent dyes (aggregation leads to quenching of fluorescence).

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**Appendix B. Calculation of the Fraction ( $\alpha$ ) of Methylene Blue in Monomer Form in an Exemplary case**

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### Appendix B. Calculation of the Fraction ( $\alpha$ ) of Methylene Blue in Monomer Form in an Exemplary case

The following equation (I), taken from Patil *et al.*, "Self Aggregation of Methylene Blue in aqueous medium and aqueous solutions of Bu<sub>4</sub>NBr and urea" (Appendix A) is found at page 4314 of the reference:

$$K = 2\alpha^2C/(1-\alpha) \quad (I)$$

wherein:  $\alpha$  is the fraction of methylene blue in monomer form,  $K$  is the dimer dissociation constant, and  $C$  is the total dye concentration ( $C = C_M + 2C_D$ , where  $C_M$  is the monomer concentration and  $C_D$  is the dimer concentration). Rearranging equation (I) and placing in standard quadratic form obtains equation (II):

$$2\alpha^2C + K\alpha - K = 0. \quad (II)$$

In the case of the current exemplary calculation,  $K = 2.9 \times 10^{-4} \text{ mol dm}^{-3}$ , and  $C = 2.2 \times 10^{-2} \text{ mol dm}^{-3}$ . The solution to equation (II) is the quadratic equation general solution with the substitutions of  $K$  and  $C$  made from directly above:

$$\alpha = \frac{-K + (K(K + 8C))^{1/2}}{4C}$$

$$\alpha = \frac{-2.9 \times 10^{-4} + ((2.9 \times 10^{-4})(2.9 \times 10^{-4} + 8(2.2 \times 10^{-2})))^{1/2}}{4(2.2 \times 10^{-2})}$$

$$\alpha = \frac{-2.9 \times 10^{-4} + ((2.9 \times 10^{-4})(2.9 \times 10^{-4} + 0.176))^{1/2}}{0.088}$$

$$\alpha = \frac{0.00686}{0.088}$$

$$\alpha = 0.078$$

As calculated in this exemplary case, the percentage of dye existing as monomer form is 7.8% and the percentage of dye existing in dimer form is correspondingly 92.2%.